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PROGRAM AND ABSTRACTS OF PAPERS CITRUS RESEARCH CONFERENCE

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December 6, 1966

Western Utilization Research and Development Division
Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in Southern California and Arizona the latest results of research on the chemistry, pharmacology, and technology of citrus fruits and their products carried on by the Utilization Research and Development Divisions of the Agricultural Research Service, U. S. Department of Agriculture. The following are participating in this year's conference.

- Western Utilization Research and Development Division:
  - Western Regional Research Laboratory (Division headquarters), 800 Buchanan Street, Albany, Calif. 94710
  - Fruit and Vegetable Chemistry Laboratory, 263 South Chester Avenue, Pasadena, Calif. 91106
- Southern Utilization Research and Development Division:
  - U. S. Fruit and Vegetable Products Laboratory, 600 Avenue S, N.W., Winter Haven, Florida 33880
- University of Arizona, Tucson, Arizona 85721
- Stanford Research Institute, 820 Mission Street, South Pasadena, Calif. 91030

PROGRAM
CITRUS RESEARCH CONFERENCE
Tuesday, December 6, 1966

Abstract on page

MORNING SESSION, 9:30 A.M.

- CHAIRMAN: *M. J. Copley, Director, Western Utilization Research and Development Division, Albany, California*
- Introductory Remarks: *Fred R. Senti, Deputy Administrator for Nutrition, Consumer and Industrial Use Research, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.*

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  Katherine Wilson and Charles Crutchfield
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- A THIN-LAYER CHROMATOGRAPHIC-COLORIMETRIC METHOD FOR THE DETERMINATION OF NARINGIN IN GRAPEFRUIT — 7
  J. F. Fisher, H. E. Nordby, and T. J. Kew (to be presented by M. K. Veldhuis)
- EVALUATION OF WURVAC — 10
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AFTERNOON SESSION, 2:00 P.M.

- CHAIRMAN: *E. A. Beavens, In Charge, Fruit and Vegetable Chemistry Laboratory, Pasadena, California*
- NEW PRODUCTS FROM DESERT GRAPEFRUIT — 12
  F. E. Nelson, J. Avery Dunn, Robert L. Brandenberger, Ralph H. Manley, Charles W. Kaufman, and Henry Tranmal
- IDENTIFICATION OF DIHYDROKAEMPFEROL AND OTHER COMPOUNDS NEW TO GRAPEFRUIT AND THEIR RELATION TO BITTERNESS — 14
  V. P. Maier and D. M. Metzler
- SOME ASPECTS OF CAROTENOID PIGMENT FORMATION IN DESERT GRAPEFRUIT — 16
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THE DETERMINATION OF LIMONIN IN ORANGE JUICE

K. W. Wilson and C. A. Crutchfield

Stanford Research Institute
South Pasadena, California

The bitter flavor which frequently appears in navel orange juice when it is heated or exposed to air is due to the presence of limonoids, especially limonin. The absence of a suitable analytical method for the determination of limonin content has severely hindered investigations of methods for prevention or removal of the bitter flavor.

At the 1965 Citrus Research Conference we described a procedure for the determination of limonin that is simple, selective, and rapid enough for routine analysis. In this procedure the limonin is isolated from the orange juice by solvent extraction and is converted to a hydroxamic acid derivative by reaction with alkaline hydroxylamine. The solution of this derivative is then acidified in the presence of ferric ion to form a colored complex which is estimated by colorimetry. This procedure has been used to measure the limonin content of freshly squeezed navel orange juice, aged juice, and commercially processed juice and concentrate. These same juices were evaluated by a taste panel to determine their degree of bitterness. There was good correlation between bitterness and the limonin content for all juices. None of the freshly squeezed juices was bitter, but as expected the early-season juices became bitter on aging. The taste threshold for limonin is between 4 and 5 p.p.m. Some preliminary data indicate that navel oranges on Trifoliate rootstock develop less bitterness than those on Troyer or Cleo rootstocks. Orange peels were analyzed for limonin content, and 78 to 98 percent of the total limonin in the fruit was found in the peel, pulp, and seeds.

In recent months the analytical method has been simplified to the point where it is rapid enough to permit analysis of twelve juice samples per day. Briefly, the steps in the analysis are as follows: (1) centrifugation of the juice, (2) solvent extraction of the limonin, (3) passage of the extract through alumina, (4) evaporation of the solvent, (5) partitioning of the residue between acetonitrile and petroleum ether, (6) addition of the color developing reagents to the acetonitrile layer, and (7) measurement of the color intensity with a colorimeter.

These studies are being carried out under a research contract with the U. S. Department of Agriculture.

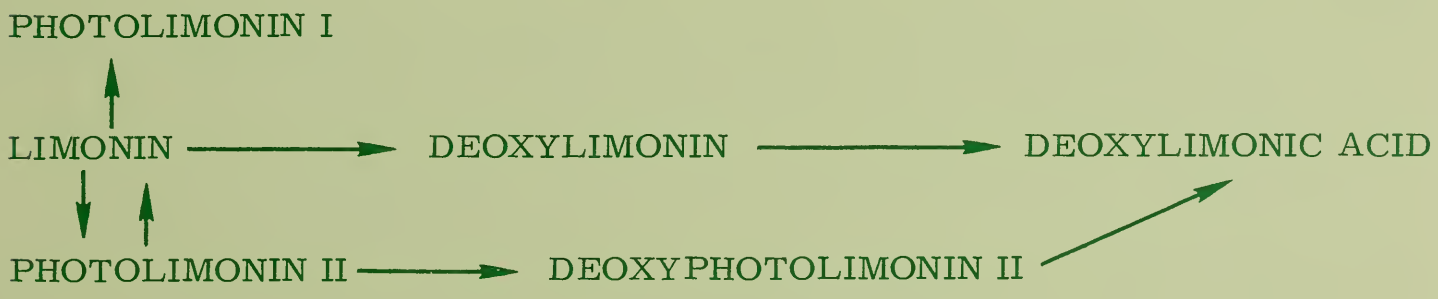
PHOTOCHEMISTRY OF THE BITTER PRINCIPLE LIMONIN

David Dreyer

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Bitterness in processed navel orange juice is generally believed to be caused by a complex triterpenoid, limonin. Selective reactions of the bitter limonin, especially to tasteless products, are of possible interest for debittering citrus juices.

I shall discuss work on the action of ultraviolet light on limonin and the properties of the photoproducts obtained. Irradiation of dioxane solutions of limonin with a mercury vapor lamp using a pyrex filter gives photolimonin I in less than 10 percent yield. The spectroscopic properties of photolimonin I suggest that the B-ring of limonin has been opened. Photolimonin I is nearly tasteless and is very closely related chemically to androbin and methyl angolensate, two naturally occurring limonoids which have been isolated from the plant family Meliaceae. When limonin in dioxane or methylene chloride is irradiated without a pyrex filter, a new photoproduct is obtained called photolimonin II that is completely tasteless. From spectroscopic studies, photolimonin II appears to be a C-8 stereoisomer of limonin. This has been confirmed by chemical studies wherein photolimonin II was converted to deoxyphotolimonin II, which on treatment with base gave deoxylimonic acid. This acid is obtained by a similar sequence from limonin.



Photolimonin II exists in a photoequilibrium with limonin. The position of equilibrium favors limonin and appears to be determined by the relative steric interactions in limonin and photolimonin II. In addition to photolimonin I and II, a photoacid of uncertain structure is also obtained, as well as a large noncrystalline fraction that appears to be general decomposition material.

RECENT STUDIES ON DIHYDROCHALCONES DERIVED FROM CITRUS FLAVANONES

R. M. Horowitz and Bruno Gentili

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The various flavanone glycosides occurring in citrus fruits contain either of the isomeric disaccharides, rutinose or neohesperidose. Rutinose is the disaccharide of hesperidin, eriocitrin, naringenin rutinoside, and isosakuranetin rutinoside. Neohesperidose is the disaccharide of naringin, neohisperidin, and poncirin. All of the flavanones that contain rutinose are tasteless, while all that contain neohesperidose are bitter. Similarly, the rutinose-containing flavanones invariably yield tasteless conversion products (as long as the rutinose part of the molecule remains intact), while the neohesperidose-containing flavanones frequently yield bitter, bittersweet, or sweet conversion products. Thus, as reported earlier, naringin and neohesperidin yield dihydrochalcones that are intensely sweet. Naringin dihydrochalcone has about the same sweetness as saccharin (on a molar basis), while neohesperidin dihydrochalcone is about twenty times sweeter. Apart from intensity of sweetness, neohesperidin dihydrochalcone is generally considered to have better taste characteristics than naringin dihydrochalcone. However, the parent compound, neohesperidin, occurs in quantity only in certain varieties of the Seville orange and is therefore costly and relatively unavailable as compared with naringin. It is of interest that a method has been devised to convert naringin to neohesperidin. The details of the procedure will be outlined.

The most available of the citrus flavanones is hesperidin. Because it is a rutinose derivative it has not figured thus far in the production of a sweet dihydrochalcone. However, a way has now been found to utilize hesperidin to prepare a sweet dihydrochalcone that seems to have promising taste characteristics. This will be discussed in detail. In addition, a summary will be presented of Dr. A. N. Booth's recent toxicity studies of the sweet dihydrochalcones.

A THIN-LAYER CHROMATOGRAPHIC-COLORIMETRIC METHOD FOR THE DETERMINATION OF NARINGIN IN GRAPEFRUIT

James F. Fisher, Harold E. Nordby, and Theo. J. Kew
(to be presented by M. K. Veldhuis)

Southern Utilization Research and Development Division
Fruit and Vegetable Products Laboratory
Winter Haven, Florida

This paper reports a relatively simplified quantitative determination of naringin in grapefruit juice employing a modification of both the Davis test and the thin-layer chromatographic system reported by Hagen and coworkers and also by Mizelle and coworkers, which separates the bitter naringin from its tasteless isomer naringenin-7β-rutinoside. The procedure is as follows:

1. A 100-ml. sample consisting of both grapefruit juice and sacs is blended for 1 min. in a Waring blender and then filtered through glass wool.

2. A 50-ml. sample of the filtrate is concentrated in vacuo at 45° C. to a viscous residue.

3. This residue is readily transferred to a 15-ml. centrifuge tube with a disposable pipette, 15 cm. long. The volume of residue in most cases has been found to be about 8 ml. The flask is rinsed with just enough water to adjust the volume in the centrifuge tube to 10 ml. The flask is then rerinsed with several portions of methyl alcohol to give a 15-ml. sample of juice, water, and methyl alcohol mixture in the centrifuge tube.

4. The contents of the centrifuge tube are well mixed and centrifuged for 5 min.

5. A 15-microliter sample of the supernatant from step 4 is streaked with a disposable micropipette, accurate to within 1 percent or less, along a 16-cm.-long pencil line drawn on a firm, nonflaky polyamide adsorbent, contained on a 20x20-cm. glass plate.

The firm, nonflaky adsorbent is prepared as follows: A mixture of 0.8 g. of rice starch, 0.4 g. of silica gel, and 9 ml. of water in a covered 20-ml. beaker is heated for 40 min. on a steam bath with occasional stirring.

Water is added during the heating as needed to prevent caking. This mixture is rinsed with 3 ml. of water into a 100-ml. beaker containing 5.5 g. of Woelm polyamide powder and 35-40 ml. of methanol. This mixture is stirred and then blended in a Waring blender micro cup for 3 min. The resulting mixture is spread as a 250-micron layer on 20x20-cm. glass plates and allowed to dry 2 hr. at room temperature before use. The pencil line does not destroy the layers and the firmness of this adsorbent layer helped to insure against mechanical loss.

6. The chromatoplate is developed twice in the 5:2, nitromethane-methyl-alcohol system reported by Hagen and co-workers to get better separation between naringin and naringenin-7β-rutinoside. Each development requires about 45 min. at 25° C. in a rectangular thin-layer chromatographic tank with a filter-paper liner.

7. After development, the plate is allowed to dry, and then lightly sprayed with 1 percent $AlCl_3$ in ethyl alcohol and exposed to UV light of 3660 Å. Both naringin and its tasteless isomer appear as bright yellow fluorescent bands.

8. The naringin band, located by its yellow fluorescence in step 7 and also by the use of an authentic naringin marker, R_f 0.31, is outlined with a pencil and the area is lightly sprayed with water. (The naringenin-7β-rutinoside has an R_f value of 0.38.) The damp polyamide-naringin area is scraped from the plate and placed in a 10x75-mm. test tube. (A small soft brush is used for brushing the scraped area clean.) The water spray prevents the formation of an electrostatic charge, which could cause a loss while scraping.

9. Into the test tube containing the scrapings 1.50 ml. of the test reagent is added from a burette. The test reagent consists of 125 ml. of methyl alcohol, 112 ml. of diethylene glycol, 13 ml. of water, and 5 ml. of 4N aqueous sodium hydroxide. The scrapings and reagent are well mixed by syringing with a disposable pipette. This procedure elutes the naringin from the polyamide and initiates the formation of a yellow color which should be allowed to develop for 10 min. before reading in a colorimeter.

10. The test tube is stoppered and centrifuged at top speed for 3 min. The clear supernatant is removed with a disposable pipette and transferred to a clean 10x75-mm. test tube.

11. The intensity of color is determined with an Evelyn photoelectric colorimeter using a blue filter (420 mμ) against a blank carried through the procedure from step 6. An adapter, to accommodate matched test tubes 10x75 mm., is placed in the tube holder. A minimum volume of 0.8 ml. of sample is required. Any colorimeter that can handle micro samples can be used.

12. The galvanometer reading, T (% transmittance), is converted to A (absorbancy) by (A = 2 - log T), from which the micrograms of naringin are determined from a standard curve. The standard curve is prepared with a standard solution of chromatographically pure naringin in methyl alcohol-water containing 1 microgram of naringin per microliter. Nine light pencil lines, each 16 cm. long, are drawn on a TLC plate composed of the same thin-layer adsorbent used in the procedure (step 5). Aliquots from 5 to 40 microliters in 5-microliter increments of the standard naringin sample are applied with disposable micropipettes along the entire length of eight of the pencil lines.

The method has been found suited for grapefruit juice, juice sacs, or other portions of the fruit. The time required is in the range of 3 to 5 hr. as opposed to several days for a previously reported procedure capable of giving comparable results.

EVALUATION OF WURVAC

A. I. Morgan, Jr. and J. L. Bomben

Western Utilization Research and Development Division
Western Regional Research Laboratory
Albany, California

The recovery of aroma from food products by most of the methods in commercial use today requires boiling at atmospheric pressure. For some products, such as citrus juices, this high temperature damages the product. The WURVAC process for recovering the volatile aroma fraction of food products uses a vacuum to obtain the desirable low temperature and a unique gas compression technique to absorb the volatile materials in any suitable solvent.

A pilot WURVAC unit has been built in our laboratory. The feed juice (120 lb./hr.) is stripped of volatiles at temperatures between 120 and 180° F. The vapor is fed to a packed stripping column where nitrogen, bled into the vacuum system, strips the volatiles immediately from the boiling condensate. The volatiles are then absorbed in a liquid-sealed vacuum pump where the nitrogen carrying the volatiles is compressed and intimately mixed with the circulating sealant, double-distilled water. By this process aroma solutions of 150- to 200-fold (volume of aroma solution/volume of feed juice) are made (Fig. 1).

With the use of gas-liquid chromatography of headspace vapors, the effects of changes in operating condition, such as temperature of evaporation, degree of concentration, reboil rate, and nitrogen bleed rate, were measured. Oil and chemical oxygen demand (COD) analyses provided additional means of evaluating the operating conditions. In the case of orange juice, evaporation of 20 percent of the feed at 180° F. gave conditions in which 90 percent of the volatiles were removed, and at the same time the juice was pasteurized. Neither the stripped juice nor the aroma solution showed any detectable damage under these conditions.

Concentrates were fortified with aroma solutions made by the WURVAC process. These concentrates were compared organoleptically and chromatographically with concentrates containing pasteurized, single-strength "cutback juice". A panel of 15 trained judges was used to evaluate the different treatments by triangle test. Reconstituted juices were judged for both aroma and flavor differences under completely independent conditions. Some of the samples were also ranked on the basis of most to least like fresh orange juice aroma. All evaluations were conducted in individual booths equipped with dim lights to eliminate influence from any possible color or appearance difference

between samples. The booths were maintained at 70 ± 2° F. with a constant flow of odor-free air.

First, samples were presented in glass-covered ruby red cups, and the panel was instructed to judge on the basis of aroma alone. After completing this evaluation the judges were presented with the same samples in 4-oz. paper cups for flavor comparison. Order of presentation was randomized and each treatment appeared as the odd sample an equal number of times. After a decision was made on the odd sample in each triangle, the judges were also asked to indicate which sample or samples had the fresher orange aroma or flavor. Each comparison for flavor and aroma was repeated twice giving a total of 30 judgments.

Those products containing the aroma solutions were judged to be more like fresh orange juice. With the use of these aroma solutions and orange oil it becomes possible to make high-quality orange concentrates of 55° Brix or more containing all the original aroma constituents, instead of a 45° Brix "cutback" concentrate containing only about 8 percent of the water-soluble aroma constituents.

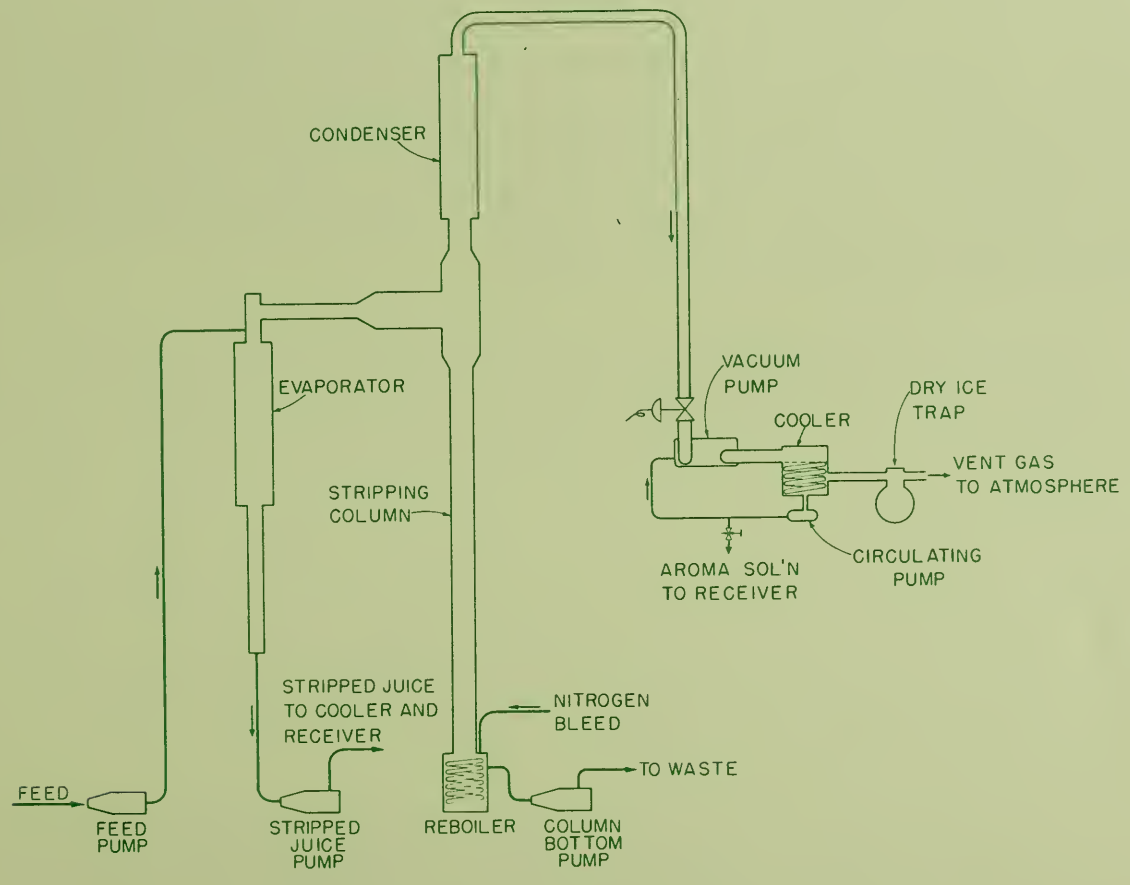


Figure I. Schematic diagram of WURVAC Process.

NEW PRODUCTS FROM DESERT GRAPEFRUIT

F. E. Nelson, J. A. Dunn, R. L. Brandenberger,
R. H. Manley, C. W. Kaufman, and Henry Tranmal

University of Arizona, Tucson

These studies are being carried out under a research contract with the U. S. Department of Agriculture. The objective is to develop new or expanded uses for desert grapefruit. Three areas are being explored: (1) piece-form, ready-to-eat fruit preparations; (2) blends of grapefruit juice with other fruit juices or purees; and (3) use of grapefruit juice as an additive to various food products.

Hand-prepared diced products, using a cube of approximately 5/8 inch, have received good consumer acceptance. Commercially available machinery is unsatisfactory for preparation of these cubes as the yield of useful pieces is too low. Studies on physical characteristics of the fruit show the bond between juice sac and membrane is the weak point. Rotary knives work satisfactorily, but pressure knives do not. Coring to remove core and central membranes is essential for consumer acceptance of the final product. A ring-type product, similar to pineapple rings, has received excellent preliminary acceptance and can be prepared much more satisfactorily. A proper package must be impervious to oxygen to avoid darkening of fruit during holding for more than a minimum time. Heating to 185° F. gives good keeping quality at room temperature and little cooked flavor.

Blends of grapefruit juice with 25 percent peach, apricot, or guava puree give products with good consumer acceptance and satisfactory keeping quality at room temperature in enameled cans. Initial acceptance is high for grapefruit juice blended with 10-15 percent strawberry, red raspberry, boysenberry, or olallieberry juice, when grapefruit juice acidity is 1.5 percent or less and sugar is added to give 14-16° Brix. These products lose color fairly rapidly and flavor somewhat less rapidly at 100° F. when held in enameled cans. At 75-80° F. they keep somewhat better and at 35° F. they are quite acceptable for several months. Glass containers increase shelf life, but data on these samples are only preliminary. Passion fruit juice is too acid to combine well with grapefruit.

Combinations of grapefruit and Thompson Seedless grape juices have not been encouraging. Cardinal, Beauty Seedless, and Scarlet grapes have proved more interesting and incorporation of some Concord juice also appears promising. The cloud must be minimized with the darker-colored grape juices to overcome a potentially undesirable consumer reaction. Considerably more study is needed in this area.

Grapefruit juice may be used in place of a portion of the vinegar in a number of food products with some increase in flavor acceptance by a significant portion of the panel. However, spoilage results when more than about 25 percent replacement is used.

Grapefruit juice added at the 10-20 percent level to peach, apricot, and guava nectars and to tomato juice improves the flavor. Using 15 percent grapefruit juice in the sirup for canning apricots and peaches resulted in flavor enhancement in preliminary studies.

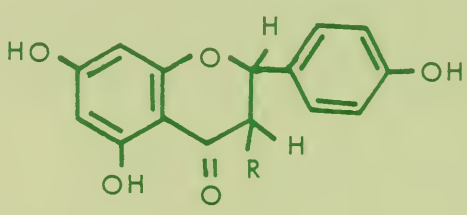
Level of the bitter principle limonin in Marsh Seedless desert grapefruit juice remained essentially constant at 8-10 p.p.m. from January through June, with Redblush juice just a little higher. This level is above the threshold for bitterness, so limonin must be considered as a contributor to bitterness in desert grapefruit.

IDENTIFICATION OF DIHYDROKAEMPFEROL AND OTHER COMPOUNDS NEW TO GRAPEFRUIT AND THEIR RELATION TO BITTERNESS

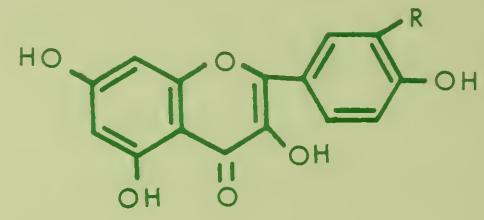
V. P. Maier and D. M. Metzler

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

It was pointed out last year that many phenolic compounds occurring at low concentrations in grapefruit remain to be identified. During the past year, with the aid of preparative thin-layer chromatographic techniques, a number of these phenolic compounds have been isolated and identified. Several are new to grapefruit and appear to have significance in the way in which the bitter flavanone glycoside naringin is transformed by the plant. One of these, dihydrokaempferol, has never before been found in citrus or other members of the Rutaceae family. Structurally, dihydrokaempferol (I) is closely related to naringenin (II) (the aglycone of naringin). In addition to structural similarity, naringenin can be converted in the laboratory into dihydrokaempferol under mild oxidizing conditions and dihydrokaempferol, in turn, can be readily oxidized to kaempferol (III). These facts suggest the



I, R = OH
II, R = H



III, R = H
IV, R = OH

sequence of reactions: naringenin → dihydrokaempferol → kaempferol as a possible pathway of naringenin transformation in grapefruit. Indirect evidence in support of this pathway is the finding that naringenin, dihydrokaempferol, and kaempferol co-occur (as glycosides) in all actively metabolizing grapefruit tissues thus far studied. Quercetin (IV), another compound new to grapefruit, is also present (as glycosides) in these tissues. Since dihydrokaempferol is known to be an efficient precursor of quercetin in buckwheat seedlings the co-occurrence of these compounds suggests that dihydrokaempferol may be a precursor of quercetin in grapefruit. Also, other compounds present in grapefruit which are related structurally to naringenin indicate the possibility of additional transformation pathways.

Thus, on the basis of compositional studies it appears that natural pathways may be present in grapefruit by which the bitter flavanone glycoside naringin or its precursors are converted into nonbitter compounds. Direct evidence based on studies with isotopically labeled compounds and cell-free enzyme systems will be required to prove whether these postulated pathways and/or others are actually operative in grapefruit.

Identification of other recently isolated compounds and their relation to the bitter taste of grapefruit will be discussed.

SOME ASPECTS OF CAROTENOID PIGMENT FORMATION IN DESERT GRAPEFRUIT

Henry Yokoyama and Michael J. White

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The distribution of carotenoids in the peel of the Marsh Seedless grapefruit was studied at various stages of maturity. In particular the hydrocarbon carotenoids were examined in detail.

The grapefruits used were obtained from a commercial grower in Indio, California, and consisted of three lots. The first collected was nearly full-sized but retained deep green color (chlorophyll) in the outer peel (flavedo). The second lot was collected when the fruit was fully matured and deep yellow; the green color had just disappeared from the flavedo. The third corresponded to the mid-season stage and the flavedo still retained the yellow color but exhibited a slight golden blush.

The peel of the Marsh Seedless grapefruit contains complex mixtures of carotenoids. The pigment extracts were initially submitted to countercurrent distribution with 100 transfers in an all-glass Craig apparatus. By means of this liquid-liquid partition method the carotenoids were fractionated into three groups: hydrocarbons, monols, and diols. Curl (J. Agr. Food Chem. 8(5):356, 1960) developed several different solvent systems and applied the method with much success to the difficult problem of separating complex carotenoid mixtures from fruits. Generally speaking, this method forms a gentle alternative to column chromatography for an introductory separation of complex mixtures of carotenoids. The grouping into isodistributive pigments are in these cases followed by adsorption chromatography to isolate the single components of the mixtures. The individual pigments were identified by comparison of chromatographic movements and visible spectra with those of authentic samples.

The green peel contains the same major colored carotenoids (β-carotene, zeaxanthin, lutein, and violaxanthin) observed in green plants. The colorless carotenoids phytoene and phytofluene occur only in trace or minor amounts in the green peel as is the case with other green tissues. This distribution pattern appears to be invariable in photosynthetic tissues and thus a regularity is detected in the carotenoid pattern in the green peel of the Marsh Seedless grapefruit. As the fruit ripens and with the disappearance of the green color or photosynthetic ability, control of carotenoid synthesis is

removed; the ripe fruit begins to exert its individuality in regard to carotenoid synthesis and accumulation. This results in a variation in the distribution pattern of carotenoids; the carotenoid pattern in the ripe fruit is no longer predictable in contrast to that observed in the green peel.

In early-season fruit at full maturity (shortly after the disappearance of the green color from the flavedo) the colorless carotenoid phytoene is still present only in trace amounts. However, a rapid buildup of the other colorless carotenoid (phytofluene) takes place. At this stage of maturity these two colorless components, phytoene and phytofluene, account for nearly 31 percent of total carotenoids.

At the midseason stage of maturity the colorless constituent phytoene constitutes the main carotenoid and accounts for nearly 51 percent of the total carotenoids. This accumulation of phytoene is significant. In the ripening fruit the development of ripe color (colored carotenoids) begins prior to the decrease in the green color (chlorophyll). This is the active period of carotenoid pigment formation. With the disappearance of the green color and further ripening of the fruit, there is a decrease in the total colored carotenoid content in the flavedo; no net synthesis of the total colored carotenoids occurs. However, during this period phytoene accumulates in the peel. This suggests that with the disappearance of green color or photosynthetic ability, an inhibition of carotenogenesis occurs in which the colorless carotenoid phytoene accumulates. That is, in the sequential pathway leading to the formation of neurosporene and lycopene via phytofluene and ζ-carotene, the stepwise dehydrogenation of phytoene to phytofluene becomes inhibited or blocked. As a consequence, in the ripe fruit no net synthesis of the total colored carotenoids takes place; instead, as the colored carotenoids undergo degradation, a decrease is observed.

ANALYSIS OF LEMON OIL

W. D. MacLeod, Jr. and Nelida Buigues

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Recent developments in the fields of gas-liquid chromatography (GLC) and thin-layer chromatography (TLC) have greatly advanced the degree to which a citrus oil such as that expressed from lemon peel can be analyzed. Temperature-programmed capillary GLC now permits the analysis of whole, cold-pressed lemon oil without any need for a prior separation into various subgroups of compounds. This was accomplished on 300 ft. x 0.010-inch I. D. stainless steel capillary tubing coated with decolorized Apiezon L grease containing 5 percent of the nonionic surfactant, nonyl phenoxypolyoxyethylene ethanol. Hydrogen flame ionization detection provided the necessary sensitivity for the analysis of 0.5 microliters of lemon oil. The sample was injected and vaporized at 120° C., then split 100:1 with the smaller portion being swept into the capillary at 40 p.s.i. pressure of helium carrier gas. After 1 min. the 300 ft. capillary column was temperature programmed from a starting temperature of 60° C. up to 150° C. at the rate of 1° C./min. The capillary coating was sufficiently stable that bleed-off of the coating during the temperature rise was inconsequential even in single column mode.

Of the more than forty volatile lemon oil components revealed, most were identified by use of a mass spectrometer in conjunction with the GLC run. Compounds identified included: α-thryene, α-pinene, octanal, camphene, sabinene, myrcene, β-pinene, α-phellandrene, α-terpinene, p-cymene, limonene, nonanal, linalool, γ-terpinene, terpinolene, citronellal, p,α-dimethylstyrene, decanal, terpinene-4-ol, neral, α-terpineol, geranial, undecanal, nonyl acetate, citronellyl acetate, neryl acetal, geranyl acetate, bergamotene, caryophyllene, and β-bisabolene.

Insofar as the nonvolatile components are concerned, the cold-pressed oils of both lemon and lime contain similar ultraviolet (UV) fluorescing compounds which may be readily separated by simple TLC into fluorescent patterns distinctly different from those of bergamot, orange, grapefruit, and tangerine cold-pressed oils. Such patterns are useful since distilled citrus oils show little UV fluorescence.

Differentiation between lemon and lime expressed oils was accomplished by a two dimensional TLC analysis. A 1-2 microliter sample of oil

was applied near the corner of a square TLC plate which was then eluted in ascending manner with 30 percent chloroform in carbon tetrachloride, then dried briefly and eluted in the second dimension with 20 percent ethyl acetate in cyclohexane.

Under UV light, known authentic cold-pressed lemon oil samples from Argentina, Arizona, coastal and inland California, Florida, Israel, and Italy all exhibited the same general pattern of fluorescent spots. This pattern was not duplicated by any other citrus oils including expressed lime oil. The TLC plate was subsequently exposed to wet HCl vapor to detect adulterants which give bright colors with HCl. Only a faint pink spot should appear. Finally, the TLC plate was sprayed with a solution of 5 percent vanillin and 30 percent phosphoric acid (85 percent aqueous) in methanol to get color reactions from the various terpenoids. Further differences between lemon and lime expressed oils were apparent in the resulting blue, green, and purple spots.

These TLC and GLC methods of analysis complement each other in providing additional criteria by which expressed lemon oil may be judged.

LIST OF PUBLICATIONS AND PATENTS*

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CITRUS BITTER PRINCIPLES. IV. OCCURRENCE OF LIMONIN IN GRAPEFRUIT JUICE. Maier, V. P. and Dreyer, D. L., J. Food Sci. 30(5):874-875, Sept.-Oct. 1965.

LEMON JUICE COMPOSITION. IV. CAROTENOID AND STEROL CONTENT. Vandercook, Carl E. and Yokoyama, Henry, J. Food Sci. 30(5): 365-368, Sept.-Oct. 1965.

RECOVERY OF FLAVORING COMPONENTS FROM ESSENTIAL OILS. Stanley, W. L., Ikeda, R. M., Vannier, S. H., and Rolle, L. A., U. S. Patent No. 3,211,740, Oct. 12, 1965.

CITRUS CAROTENOIDS. IV. THE ISOLATION AND STRUCTURE OF SINTAXANTHIN. Yokoyama, Henry and White, Michael J., J. Org. Chem. 30(11):3994-3996, Nov. 1965.

THE CONSTITUTION OF NOOTKATONE, NOOTKATENE AND VALENCENE. MacLeod, William D., Jr., Tetrahedron Letters No. 52:4779-4783, Dec. 1965.

NOOTKATONE, GRAPEFRUIT FLAVOR AND THE CITRUS INDUSTRY. MacLeod, W. D., Jr., The Calif. Citrograph 51(3):120-123, Jan. 1966.

LEMON JUICE COMPOSITION. V. EFFECTS OF SOME FRUIT STORAGE AND PROCESSING VARIABLES ON THE CHARACTERIZATION OF LEMON JUICE. Vandercook, Carl E., Rolle, Laurence A., Postmayr, H. L., and Utterberg, R. A., J. Food Sci. 31(1):56-62, Jan.-Feb., 1966.

LONG RANGE PROTON SHIELDING IN C-GLYCOSYL COMPOUNDS: STRUCTURE OF SOME NEW C-GLYCOSYLFLAVONES. Horowitz, R. M. and Gentili, B., Chem. and Indus. 12:625-627, April 9, 1966.

---

*Reprints are available at the addresses indicated; patents are available only by purchase at 50¢ a copy from the U. S. Patent Office, Washington, D. C. 20250.

CITRUS BITTER PRINCIPLES. V. BOTANICAL DISTRIBUTION AND CHEMOTAXONOMY IN THE RUTACEAE. Dreyer, David L., Phytochem. 5(3):367-378, May 1966.

COLLABORATIVE STUDY ON DETERMINATION OF l-MALIC ACID IN LEMON JUICE. Yokoyama, Henry, J. Assoc. Off. Analyt. Chem. 49(3): 621-623, June 1966.

LEMON OIL ANALYSIS. I. TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY. MacLeod, Wm. D., Jr. and Buigues, Nelida M., J. Food Sci. 31(4):588-590, July-Aug. 1966.

LEMON OIL ANALYSIS. II. GAS-LIQUID CHROMATOGRAPHY ON A TEMPERATURE-PROGRAMMED, LONG, OPEN TUBULAR COLUMN. MacLeod, Wm. D., Jr., McFadden, W. H., and Buigues, Nelida M., J. Food Sci. 31(4):591-594, July-Aug. 1966.

CITRUS BITTER PRINCIPLES. VI. ICHANGIN. Dreyer, David L., J. Org. Chem. 31:2279-2281, 1966.

LEMON JUICE COMPOSITION. IDENTIFICATION OF MAJOR PHENOLIC COMPOUNDS AND ESTIMATION BY PAPER CHROMATOGRAPHY. Vandercook, C. E. and Stephenson, R. G., J. Agr. Food Chem. 14(5):450-454, Sept.-Oct. 1966.

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THE STRUCTURE OF β-SINENSAL. Flath, R. A., Lundin, R. E., and Teranishi, R., Tetrahedron Letters No. 3:295-302, Jan. 1966.

DIALYZER CONCENTRATES BEVERAGES. Popper, K., Camirand, W. M., Nury, F., and Stanley, W. L., Food Engin. 36(4):102, 104, April 1966.

METHODS OF USING FLAVYLIUM COMPOUNDS FOR FOOD COLORING. Jurd, L., U. S. Patent No. 3,266,903, Aug. 16, 1966.

CHARACTERIZATION OF FOAMS FOR FOAM-MAT DRYING. LaBelle, R. L., Food Technol. 20(8):89-94, August 1966.

VACUUM STRIPPING OF AROMAS. Bomben, John L., Kitson, John A., and Morgan, A. I., Jr., Food Technol. 20(9):125-128, Sept. 1966.

ORANGE COMPOUNDS. VOLATILES FROM ORANGES. OXYGENATED COMPOUNDS IDENTIFIED BY INFRARED, PROTON MAGNETIC RESONANCE, AND MASS SPECTRA. Teranishi, Roy, Lundin, R. E., McFadden, W. H., Mon, T. R., Schultz, T. H., Stevens, K. L., and Wasserman, Jan, J. Agr. Food Chem. 14(5):447-449, Sept.-Oct. 1966.

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FLAVONES OF THE NEUTRAL FRACTION OF THE BENZENE EXTRACTABLES OF AN ORANGE PEEL JUICE. Swift, L. J., J. Agr. Food Chem. 13(5):431-433, Sept.-Oct. 1965.

ISOLATION AND SPECTRAL CHARACTERIZATION OF COUMARINS IN FLORIDA GRAPEFRUIT PEEL OIL. Fisher, J. F. and Nordby, H. E., J. Food Sci. 30(5):869-873, Sept.-Oct. 1965.

CONVERSION OF VALENCENE TO NOOTKATONE. Hunter, G. L. K. and Brogden, W. B., Jr., J. Food Sci. 30(5):876-878, Sept.-Oct. 1965.

COMPOSITION OF ORANGE JUICE CLOUD. Scott, W. C., Kew, T. J., and Veldhuis, M. K., J. Food Sci. 30(5):833-837, Sept.-Oct. 1965.

LEVEL FOR CHROMATOGRAPHIC COLUMNS. Moshonas, M. G. and Hunter, G. L. K., Chemist-Analyst 54:124, Oct. 1965.

5-HYDROXYMETHYLFURFURAL IN STORED FOAM-MAT ORANGE POWDERS. Berry, R. E. and Tatum, J. H., J. Agr. Food Chem. 13(6):588-590, Nov.-Dec. 1965.

PROCEEDINGS OF THE CITRUS PROCESSING CONFERENCE. U. S. Dept. Agr., ARS 72-43, 23 pp., Jan. 1966.

ANALYSIS OF ALCOHOLS IN ESSENTIAL OILS OF GRAPEFRUIT, LEMON, LIME AND TANGERINE. Hunter, G. L. K. and Moshonas, M. G., J. Food Sci. 31(2):167-171, April 1966.

STORAGE STUDIES ON FOAM-MAT-DRIED GRAPEFRUIT POWDER. Berry, R. E., Bissett, O. W., Wagner, C. J., Jr., and Veldhuis, M. K., Food Technol. 20(4):177-178, April 1966.

TWO NEW COUMARINS FROM GRAPEFRUIT PEEL OIL. Fisher, J. F. and Nordby, H. E., Tetrahedron 22:1489-1493, April 1966.

PREVENTION OF FOAM IN JUICE FROM RECONSTITUTED CITRUS POWDERS. Berry, R. E., Bissett, O. W., and Wagner, C. J., Jr., Proc. Florida State Hort. Soc. 78:202-207, May 1966.

RAPID ESTIMATION OF RECOVERABLE OIL IN CITRUS JUICES BY BROMATE TITRATION. Scott, W. C. and Veldhuis, M. K., J. Assoc. Off. Analyt. Chem. 49:628-633, June 1966.

SEASONAL VARIATION OF NARINGIN AND CERTAIN OTHER FLAVANONE GLYCOSIDES IN JUICE SACS OF TEXAS RUBY RED GRAPEFRUIT. Hagen, R. E., Dunlap, W. J., and Wender, S. R., (Chemistry Department, University of Oklahoma, Norman), J. Food Sci. 31(4):542-547, July-Aug. 1966.

ANALYSIS OF COLD-PRESSED ORANGE OIL PARAFFIN WAXES. Hunter, G. L. K. and Brogden, W. B., Jr., Phytochemistry 5: 807-809, 1966.

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